Maternal Diesel Inhalation Increases Airway Hyperreactivity in Ozone-Exposed Offspring

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Air pollutant exposure is linked with childhood asthma incidence and exacerbations, and maternal exposure to airborne pollutants during pregnancy increases airway hyperreactivity (AHR) in offspring. To determine if exposure to diesel exhaust (DE) during pregnancy worsened postnatal ozone-induced AHR, timed pregnant C57BL/6 mice were exposed to DE (0.5 or 2.0 mg/m^3) 4 hours daily from Gestation Day 9–17, or received twice-weekly oropharyngeal aspirations of the collected DE particles (DEPs). Placentas and fetal lungs were harvested on Gestation Day 18 for cytokine analysis. In other litters, pups born to dams exposed to air or DE, or to dams treated with aspirated diesel particles, were exposed to filtered air or 1 ppm ozone beginning the day after birth, for 3 hours per day, 3 days per week for 4 weeks. Additional pups were monitored after a 4-week recovery period. Diesel inhalation or aspiration during pregnancy increased levels of placental and fetal lung cytokines. There were no significant effects on airway leukocytes, but prenatal diesel augmented ozone-induced elevations of bronchoalveolar lavage cytokines at 4 weeks. Mice born to the high-concentration diesel–exposed dams had worse ozone-induced AHR, which persisted in the 4-week recovery animals. Prenatal diesel exposure combined with postnatal ozone exposure also worsened secondary alveolar crest development. We conclude that maternal inhalation of DE in pregnancy provokes a fetal inflammatory response that, combined with postnatal ozone exposure, impairs alveolar development, and causes a more severe and long-lasting AHR to ozone exposure.

Keywords: diesel; fetal inflammation; ozone; airway hyperreactivity

Increasing asthma prevalence among children in industrialized nations has stimulated a number of investigations focused on early life exposures to air pollutants (1). Maternal exposures to a variety of inhaled atmospheric pollutants, including side and mainstream tobacco smoke, diesel exhaust (DE), and ozone, have been linked in epidemiologic studies to asthma and other respiratory diseases in children (2–4). Animal models aimed at deciphering the mechanisms by which maternal exposures during pregnancy are linked to childhood asthma have largely focused on pathways relevant to allergic asthma (5).

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CLINICAL RELEVANCE

Maternal murine exposure to diesel inhalation provokes a fetal inflammatory response that worsens the effects of neonatal chronic ozone on airway hyperreactivity. This effect persists to adulthood, despite cessation of ozone exposure. Prevention of maternal exposure to air pollution during pregnancy may reduce the risk for childhood asthma.

In addition to allergic sensitization, *in utero* exposure to air pollutants may also affect nonspecific pulmonary responses, such as airway hyperreactivity (AHR) (6). It has already been shown that fetal pulmonary inflammatory challenges are linked with impaired postnatal alveolar development (7–9), which affects airway stability and AHR (10). Previous studies have shown that maternal exposure to DE during pregnancy modified mouse placental cytokines relevant to airway inflammation (11), and lowered the threshold for AHR in ovalbumin-sensitized offspring (12).

We have previously shown that a standardized urban particulate delivered by oropharyngeal aspiration during pregnancy induced placental inflammatory cytokine expression and worsened neonatal ozone-induced AHR (6), without apparent effects on postnatal lung development. Because inhalation of materials is a highly relevant route of exposure and obviates the need for stressful anesthesia during pregnancy, we conducted studies to determine whether or not inhalation of DE could exacerbate ozone-induced AHR in the offspring. Timed pregnant mice were exposed to inhaled DE at airborne concentrations relevant to human exposures, as recently described in detail (13). A subset of offspring were exposed to intermittent ozone beginning at birth for 4 weeks, as previously described (6), whereas others recovered in room air for an additional 4 weeks to determine if postnatal exposure effects persisted to adulthood. Parallel studies were also conducting using mice exposed by oropharyngeal aspiration to compare the different exposure routes and determine whether this method could be validated for other materials, such as size-fractionated ambient particulate matter (PM) from different locations where exposures by inhalation would not be practical. Some of the findings described here have been published in the form of an abstract (14).

MATERIALS AND METHODS

Reagents were from Sigma (St. Louis, MO) except where noted. All procedures were approved by institutional animal care and use committees. C57BL/6 mice were obtained from Charles River Laboratories (Raleigh, NC).

Animal Exposures: DE

Mice were housed in pathogen-free vivarium conditions. Time-mated females were exposed to air or 0.5 or 2.0 mg/m^3 of DE for 4 hours/day from Gestation Day (GD) 9 to GD17 (*see* details in the online supplement).

DE Particle Oropharyngeal Aspiration

DE particles (DEPs) were collected from a single-cylinder diesel generator engine (*see* the online supplement). Additional time-mated females were treated with DEP delivered by oropharyngeal aspiration instead of by inhalation. Beginning on GD3, mice were placed under 0.5% isoflurane anesthesia and suspended by their frontal incisors. The tongue was extended with smooth forceps and the suspension of DEPs or vehicle (PBS [pH 7.2], 0.05% Tween 20, 50 μl) was pipetted into the oropharynx (15). Mice received 50 μg suspended in 50 μl vehicle twice weekly for 3 weeks (6).

Fetal Tissue Cytokine Analysis

Fetuses and placentas were obtained at GD18 by hysterectomy under sodium pentobarbital anesthesia (250 mg/kg intraperitoneal). Fetuses were placed on ice and then decapitated. Placentas and fetal lungs were snap frozen under liquid nitrogen. Multiplex cytokine analysis was performed on fetal lung and placenta homogenates and normalized to total protein (16).

Postnatal Exposures

After exposure to diesel or filtered air from GD9–17, dams were transported from the U.S. Environmental Protection Agency inhalation exposure facilities (6 miles) to Duke University, where they were housed separately in a satellite animal facility, and gave birth between GD18 and GD20. Beginning Postnatal Day 2, dams and pups were exposed to filtered air or ozone (1 ppm) for 3 hours per day, 3 days per week for 4 weeks, as previously described (6). Juvenile mice underwent a recovery period for 4 weeks under routine vivarium conditions before measurement of pulmonary mechanics.

Lung Inflammation

At 4 weeks of age, air- and ozone-exposed mice born to air- or DE-exposed dams underwent bronchoalveolar lavage (BAL) and cellular analysis as previously described in detail (6). In a subset, BAL fluid (BALF) cytokine concentrations were determined as described previously here.

Pulmonary Mechanics

At 4 or 8 weeks of age, mice from each treatment group underwent pulmonary mechanics measurements using the forced oscillatory ventilation method (flexiVent; SCIREQ, Montreal, PQ, Canada) (6) before and after nebulized methacholine challenge at 0, 100, 250, and 500 mg/ml (4 wk) or 0, 12.5, 25, and 100 mg/ml (8 wk).

Airway Smooth Muscle

Sections from air- and ozone-exposed mice born to DE- or filtered air–exposed dams were immunolabeled with anti–α-smooth muscle actin–Cy3 and imaged (17) (*see* details in the online supplement).

Airway Mucous Metaplasia

Two random sections from four mice in each treatment group were stained with periodic acid–Schiff and Alcian blue. A mucous metaplasia score was assigned to large and small airways in each section and the means of individual mean scores were compared (*see* details in the online supplement).

Alveolar Morphology

Alveolar volume and surface density, and secondary alveolar septal crest density, were measured in random sections obtained from air- and ozone-exposed mice born to DE or filtered air–exposed dams ($n = 5$/group) (18) (*see* details in the online supplement).

Statistical Analysis

Intergroup differences were identified using ANOVA. *Post hoc* comparisons were made using Tukey's highly significant difference test (KaleidaGraph version 4.1; Synergy Software, Reading, PA). Statistical significance was accepted at P less than 0.05.

RESULTS

DE Chamber Concentrations

Table E1 in the online supplement describes the average DE concentration and particle size data. There was no evidence of significant nuclei or coarse particle modes. Concentrations of carbon monoxide, nitric oxide, and nitrogen dioxide were all below threshold levels of concern.

Effect of Maternal Pulmonary DE and DEP Exposure on Fetal Cytokine Expression

Inhaled DE exposure induced 2- to 4-fold increases in all measured cytokines in both placenta and fetal lung at GD18 compared with vehicle or air exposure (Figure 1). Proinflammatory cytokine increases in fetal lung and placenta were not always parallel, with higher increases in fetal lung than in placenta. Nearly parallel findings were observed in fetal tissues obtained from dams treated with DEP or vehicle instillation, with a few exceptions. In fetal tissues from DEP- or vehicle-treated dams, regulated upon activation, normal T cell expressed and secreted (RANTES) baseline levels were higher, and there were no disparities between expression of RANTES in lung and placenta. In contrast with tissues from DE exposure, keratinocyte-derived chemokine was elevated only in the placenta. Fetal lung eotaxin concentrations from DEP-treated dams were lower than from DE-treated dams.

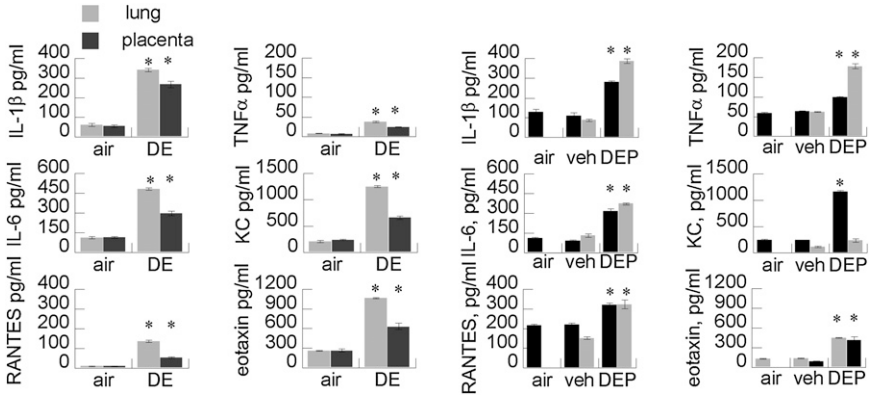

Figure 1. Effects of maternal diesel exhaust (DE) exposure or maternal DE particle (DEP) oropharyngeal aspiration versus air (DE control) or vehicle (Veh; DEP control) on fetal cytokines at Gestation Day 18. Data are means of 10 animals per group (±SEM); *$P < 0.001$ versus air or vehicle control. KC, keratinocyte-derived chemokine; RANTES, regulated upon activation, normal T cell expressed and secreted.

Effect of Maternal Pulmonary DE Exposure with Postnatal Filtered Air or Ozone Exposure on Survival and Somatic Growth

There was no effect of diesel exposure on litter size (8–10 across all groups). All ozone-exposed groups had decreased body weight at Day 28, as did pups born to dams exposed to diesel inhalation alone at 0.5 mg/m^3. Maternal exposure to DE at 2.0 mg/m^3 had no effect on body weight in pups that were exposed to postnatal air (Figure 2).

Effect of Maternal Pulmonary DE Exposure with Postnatal Filtered Air or Ozone Exposure on Pulmonary Inflammation at 4 Weeks after Birth

There were no statistically significant increases in BALF leukocytes regardless of pre- or postnatal treatment (Figure 3). BALF cells were predominantly macrophages (>97%) in all treatment groups, with no effects of treatment on the proportion of neutrophils or eosinophils (both <2% in all groups [data not shown]). Despite the absence of treatment effects (pre- or postnatal) on BALF inflammatory cells, there were significant elevations in proinflammatory cytokine concentrations in BALF from ozone-exposed animals, which were further increased in those born to DE-exposed dams.

Effect of Maternal DE and DEP Exposure on Ozone-Induced Changes in Pulmonary Mechanics: 4 and 8 Weeks

When we assessed AHR as a change in total respiratory system resistance in response to increasing doses of nebulized methacholine, postnatal ozone exposure was found to increase AHR. This AHR response, which is typically identified as an asthma phenotype, was more robust in the pups born to dams exposed to DE inhalation at 2.0 mg/m^3, but not 0.5 mg/m^3 (Figure 4A). Inhaled DE exposure in dams had no effect on AHR in air-exposed offspring. With respect to the changes in total respiratory resistance, there were only modest effects of ozone exposure with or without DE on large airway (Newtonian) resistance (data not shown). In pups that were allowed to recover after ceasing ozone or air exposure and advanced to adulthood (8 wk of age at evaluation), we found that sensitivity to nebulized methacholine was increased in all treatment groups (Figure 4C), and that the ozone-exposed pups (even without maternal DE exposure) had persistent AHR to methacholine, which was again significantly augmented in pups born to dams that were treated with 2.0 mg/m^3 DE. We found parallel effects in 4-week-old offspring born to dams that received oropharyngeal aspiration of DEP during pregnancy (Figure 4B), which also persisted in the 8-week-old recovered mice (Figure 4C). None of the treatments had effects on baseline tissue damping,

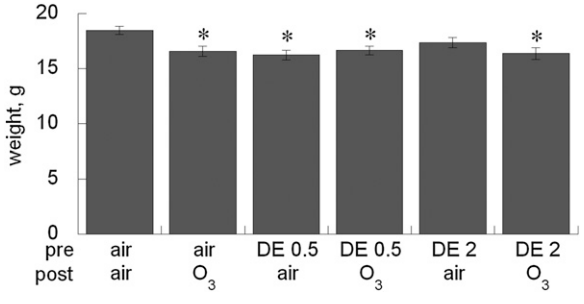

Figure 2. Effect of prenatal air or DE (0.5 or 2.0 mg/m^3) with or without postnatal air or ozone on body weight at Postnatal Day 28 (n = 25–30 animals/group; mean ± SEM); *P < 0.05 versus preair/postair control.

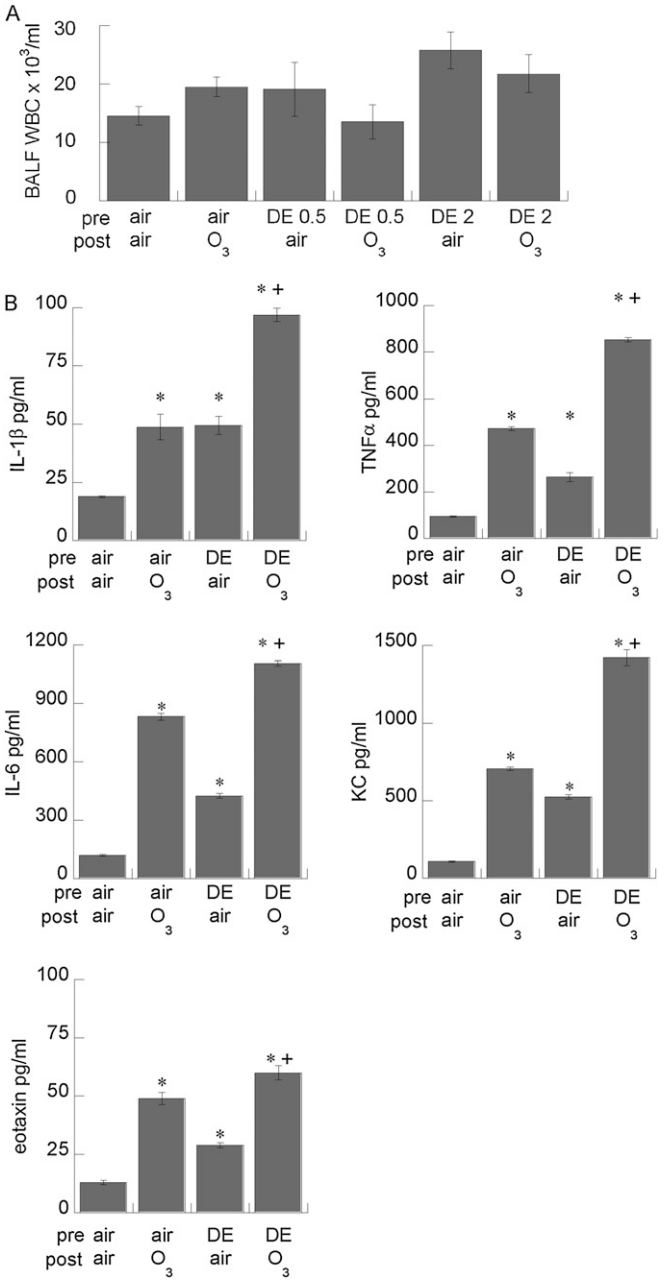

Figure 3. (*A*) Effect of prenatal air or DE (0.5 or 2.0 mg/m^3) with or without postnatal air or ozone on bronchoalveolar lavage leukocyte counts at P28. Data are means (±SEM) (n = 10/group). There were no significant differences between treatment groups. (*B*) Effect of prenatal air or 2.0 mg/m^3 DE with or without postnatal air or ozone on cytokine concentrations in bronchoalveolar lavage fluid. Data are means of five animals per group (±SEM). *P < 0.05 versus preair/postair control; ^+P < 0.05 versus preair/postozone.

compliance, or elastance at either age, but compliance increased with age, with reciprocal effects on tissue damping and elastance (Figures 4D–4F), as expected (19).

Effect of Maternalde Exposure with Postnatal Filtered Air or Ozone Exposure on Small Airway Structure

There were no obvious effects on qualitative α-smooth muscle actin labeling around small (75–200 μm diameter) or large (>200 μm) airways (*see* Figure E1). As in our previous study

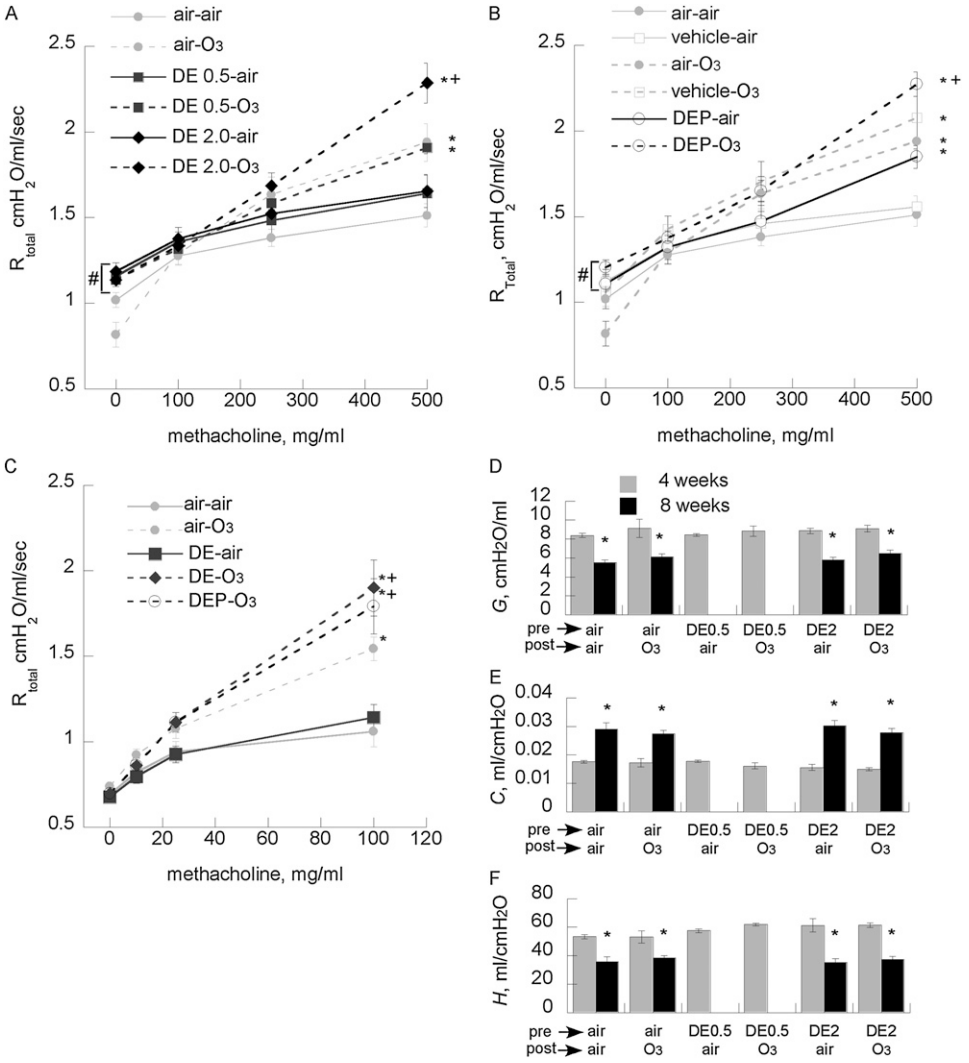

Figure 4. (*A–C*) Total respiratory system resistance in response to nebulized methacholine in mice born to dams exposed to inhaled DE (*A*) or aspirated DEP (*B*) at 4 weeks, after postnatal air or O_3 exposure, and (*C*) 4 weeks after cessation of air or O_3. *Gray lines*, prenatal air exposure; *black lines*, prenatal diesel exposure (ambient DE: *closed squares*, 0.5 mg/m^3; *closed diamonds*, 2.0 mg/m^3; *open circles*, instilled DEP). *Solid lines*, postnatal air exposure; *dashed lines*, postnatal ozone exposure. Data are means of 10–12 animals/group (±SEM). *$P < 0.05$ versus air/air control; $^+P < 0.05$ versus air/O_3; $^\#P < 0.05$, prenatal DE versus prenatal air. (*D–F*) Effects of pre- and postnatal exposures on (*D*) tissue damping, (*E*) respiratory system compliance, and (*F*) tissue elastance, at 4 (*shaded bars*) and 8 (*closed bars*) weeks. Data are means of 10–12 animals/group (±SEM); *$P <$ 0.05 versus 4 weeks. *C*, respiratory system compliance; *G*, tissue damping; *H*, tissue elastance.

(6), the α-actin–positive smooth muscle surrounding small airways was typically discontinuous. There were no treatment effects on airway epithelial cell mucous metaplasia scores (Figure E2).

Effect of Maternal DE Exposure with Postnatal Filtered Air or Ozone Exposure on Alveolar Development

There were no effects of treatment on alveolar volume density (estimates alveolar number) or surface density at Postnatal Day 28 (Figures 5A and 5B). However, secondary alveolar crest density, an inflation-independent measurement of alveolar development, was significantly worse in mice born to diesel-exposed dams and subsequently exposed to ozone (Figure 5C). Prenatal diesel or postnatal ozone alone had no significant effect on secondary crest density. Elastin-stained septal crests are clearly visible in representative photomicrographs from each treatment group (Figures E3A–E3C) except the prenatal DE, postnatal ozone group (Figure E3D).

DISCUSSION

The relationship between maternal environmental exposures during pregnancy and the increased risk for the development of asthma in children has prompted a number of investigations in preclinical models largely focused on allergic asthma (5). In addition, reduced lung growth has been associated with children residing in communities with high oxidant air pollution levels, suggesting that structural changes may contribute (20). Because ozone- and traffic-related particle exposures are also linked with asthma exacerbations in children (although possibly through

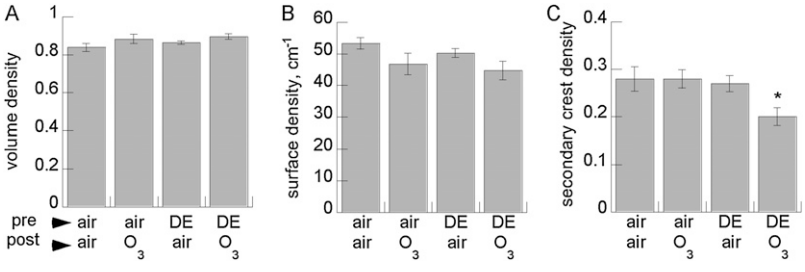

Figure 5. Effect of prenatal air or DE (2 mg/m^3) exposure plus postnatal air or ozone exposure on (*A*) alveolar volume density, (*B*) alveolar surface density, and (*C*) secondary alveolar crest density at 4 weeks. Data are means of five animals/group (±SEM); *$P < 0.05$ versus prenatal DE/postnatal air.

different intrinsic mechanisms than allergic asthma), we wanted to determine if maternal exposure to air pollutants in the absence of postnatal allergic stimuli led to lung dysfunction in offspring. As a first step to support this hypothesis, we previously demonstrated that aspiration of a standardized urban particulate provoked increased placental inflammatory cytokine expression and exacerbated ozone-induced AHR in juvenile-aged murine offspring (6).

In the present study, we refined and expanded our approach and exposed pregnant mice by the normal physiological route (inhalation) to freshly generated internal combustion engine DEPs at 0.5 or 2.0 mg/m^3 (21), and found concentration-dependent augmentation of ozone-induced AHR at 4 weeks of age, which persisted to adulthood. The magnitude of the AHR effect in 4-week-old, ozone-exposed mice born to DE- or DEP-exposed dams is comparable to the increment in AHR that we previously reported in ozone-exposed mice born to dams treated with instilled St. Louis particle (6), and with the magnitude of AHR increment measured by forced oscillometry in ovalbumin-sensitized 22-day-old BALB/c mice born to bisphenol-A–treated dams (22).

Exposure to DE by inhalation or oropharyngeal aspiration of collected DEPs led to elevations of both placental and fetal lung inflammatory cytokines. Oropharyngeal aspiration of pollutants to pregnant mice just before term (GD17) was sufficient to enhance allergic sensitization in offspring (12), but chronic maternal inhalation of DE did not enhance ovalbumin challenge–induced AHR in offspring (BALB/c strain) (13). Others have shown that maternal DE exposure (0.3–3.0 mg/m^3; beginning at GD2, continued through GD13) in mice (ICR strain) led to high rates of placental/fetal resorption, but, in general, depressed IL-1β, and induced IL-2, -4, and -5 mRNA in placentas obtained at GD14 (11). We also observed high rates of resorption in preliminary studies (data not shown) using a similar exposure duration, so we chose to perform maternal DE exposures beginning on GD7 and continuing to GD17, which has been shown to lessen fetal resorption (to ~20%) in BALB/c mice exposed to a 3.1-mg/m^3 diesel concentration (13). Placental and fetal lung cytokine expression were examined at the GD18 time point, and showed significant elevations of IL-1β and TNF-α along with other cyto-/chemokines. These results contrast with the findings of Fujimoto and colleagues (11), possibly due to the later stage of pregnancy at which our measurements were taken. Because only one time point was examined, however, the temporal nature of these responses is still unknown.

The diesel exposure levels (0.5 and 2.0 mg/m^3) are comparable to occupational exposures in mine workers to airborne particulates where DE is the principal source (~1.28 mg/m^3) and for commuters in public transit depots where levels have been reported as high as 0.75 mg/m^3 (23). In mechanistic human studies, liquid extracts containing 100–300 μg of industrial PM have been delivered into a single lung segment (24), or sprayed directly onto the nasal mucosa at doses of DE particulate between 150 and 1,000 μg (25). By comparison, and based upon dosimetric calculations of deposition fraction and minute ventilation for mice, the cumulative (4 h/d × 11 d) diesel particulate lung burdens in the dams were expected to range between 4.4 μg for the low-exposure (0.5 mg/m^3) and 17.6 μg for the high-exposure (2.0 mg/m^3) levels, respectively.

Fetal inflammatory signaling has emerged as a common theme in a number of model systems designed to examine *in utero* effects on lung development, such as impaired alveolar development. Direct fetal exposure to proinflammatory cytokines or endotoxin impairs alveolar development in lambs, for example (*see* Ref. 26 for review). Early life inflammatory exposures may also affect later growth and repair capacities, increasing vulnerability to a "second hit," such as ozone. However, the mechanisms by which maternal inflammatory responses are transduced to the fetus are unclear. Evidence for transplacental cytokine transport is decidedly mixed with a paucity of direct labeling studies. Maternally ingested diesel particles are associated with oxidative DNA damage in embryos (27), but, to our knowledge, no detection of particles in embryos/fetuses has been reported. Elegant, albeit indirect, studies using transgenic mice lacking cytokine receptors for IL-4 or IL-13, pregnant with wild-type (heterozygous) offspring, showed that maternal injection with receptor ligands had no effect on cytokine receptor signaling in fetal tissues, strongly suggesting that IL-4 and IL-13 do not cross the placenta (28). Instead, circulating maternal signaling molecules (e.g., cytokines, "alarmins," hyaluronan) may stimulate placental production of proinflammatory cytokines that contribute to altered development in the fetus and offspring (29). Exposures in early pregnancy could also have effects on fetal imprinting, altering immune responses by epigenetic mechanisms, a pathway of considerable interest, given the direct evidence in experimental asthma (30, 31) and the suggestive associations reported in human asthma (32).

We found that, although diesel exposure was sufficient to provoke fetal inflammatory cytokine expression, postnatal ozone exposure was required to impair alveolar development, which contributes to alveolar instability, resulting in small airway closure in response to agonist challenge, such as methacholine. This structural impairment accompanied the increase in AHR. Alveolar derecruitment is increasingly recognized as a potential contributor to AHR (10). Other model systems that produce mild to moderate impairments of alveolar development (33) with postnatal oxidative stress also demonstrate increased AHR (34). The magnitude of the decrement in secondary alveolar crest number in our present study in 28-day-old mice (diesel/ozone) is comparable to the decrement previously reported in hyperoxia-exposed newborn rats (33). Airway-targeted (Clara cell secretory protein promoter) inflammation in IL-11–overexpressing mice also produced impaired alveolar development and decreased numbers of alveolar attachments to small airways in mice that demonstrated AHR in response to methacholine (35). Because AHR and, indeed, asthma are complex phenotypes, it is likely that multiple pathophysiologic pathways contribute to airway instability after bronchoprovocation, and we cannot be certain that impaired alveolar development is the dominant contributor in these studies.

Indeed, ozone exposure effects on persistent, as opposed to acute, AHR in mice have been studied chiefly in the context of ozone effects on pulmonary adaptive (augmentation) or innate (impairment) immunity, with relatively few studies of neonatal or juvenile ozone exposure. These were chiefly concerned with inflammatory responses (36, 37), rather than AHR. We previously observed increased inflammatory cytokines in lungs of mouse pups born to PM-instilled dams and exposed to ozone for 4 weeks (6), and found similar elevations in BALF cytokines in ozone-exposed mice born to DE-treated dams, but no corresponding effects on BALF leukocytes. As in our prior study, we found no differences between treatment groups in histologic evidence of inflammation in small airways (Figure E2). The elevations in BAL proinflammatory cytokines in the absence of corresponding alterations in BAL leukocytes may represent a phenotypic change in local airway epithelial responsiveness, but, in the setting of chronic ozone exposure, may be accompanied by desensitization of leukocyte cytokine/chemokine receptors. It may also be that ozone has subtle effects on tissue leukocytes near airways or recruited to airways that could indirectly affect AHR through interaction with neural signaling. In a mouse model of allergic airways, dendritic cells have been

found to have close proximity to airway nerves (38, 39), but the functional significance is unknown. In the guinea pig, eosinophils have been shown to interfere with termination of airway neuromuscular signaling (40), but, to our knowledge, this mechanism has not been shown in other species.

Although inhaled DE represents the physiologic route of exposure, oropharyngeal aspiration ensures accurate dose deposition to the lower airways (15), so we conducted parallel studies with this method to deliver previously collected DEPs. Results were comparable for both delivery modes (inhalation and instillation), with placental and fetal lung cytokine levels showing elevations compared with those obtained from air- or vehicle-treated dams, with few exceptions. Baseline RANTES concentrations were higher in fetal tissues after maternal DEP than DE treatment, but there were also elevations in contemporaneous air and vehicle controls. Differences between placental and fetal lung inflammation provoked by maternal DE and DEPs may be related to differences in deposition efficiency for the two methods, or to differences in the chemical characteristics (fresh versus aged) (41). In addition, the exposure regimens differed (continuous versus intermittent).

In an attempt to address further the potential for persistence of the pulmonary mechanical effects at a time when mice have advanced to an adult stage of lung development, and without continued exposure to ozone, we performed an additional set of experiments to determine the persistence of the AHR response to postnatal ozone. This led to our unique observation that the ozone-enhanced airway reactivity to methacholine did not reverse after a 4-week recovery period (from juvenile stage of 4 wk to adult stage of 8 wk) (Figure 4C). Consistent with the treatment groups analyzed at 4 weeks, recovered mice born to dams treated with DE or DEPs exhibited augmented ozone-induced AHR to methacholine as adults at 8 weeks. To our knowledge, previous studies of murine neonatal ozone exposure have not examined effects on the persistence of AHR to adulthood. Neonatal rats exposed to ozone (2 ppm) developed AHR and increased substance P–positive small airway nerves upon rechallenge with acute ozone at Postnatal Day 28 (42). This postnatal window of vulnerability to ozone exposure is consistent with our observations. If AHR responses persist beyond a few days after cessation of ozone exposure in children, this would be of importance to the understanding of the interactions between ozone exposure and asthma exacerbations.

In summary, we conclude that diesel inhalation during pregnancy provokes a fetal inflammatory response that, when combined with intermittent postnatal ozone exposure, is associated with impaired alveolar development and an increase in ozone-induced AHR in offspring that persists to adulthood, despite cessation of ozone exposure. Although we did not observe other structural changes in lung parenchyma, we speculate that functional alterations in neural signaling or airway smooth muscle function could also be contributors. Maternal exposures to traffic-related air pollutants may have long-lasting effects on the susceptibility of offspring to ozone-provoked AHR that persist to adulthood.

Author disclosures are available with the text of this article at www.atsjournals.org.

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